IV. CLINICAL STUDIES: SPECIFIC REQUIREMENTS

A. Plan clinical studies to prove clinical utility and safety and effectiveness.

1. Prove all diagnostic claims and specific parameters important for the clinical utility of the device

2. Each manufacturer must have three testing sites and educate each of the investigators to the dangers in the use of incorrect reference data. This is a common cause of incorrect interpretation of MSAFP testing measurements by physicians and laboratorians, it is essential that the manufacturer monitor each of its testing laboratories to establish the testing laboratories' own reference data and/or demonstrate that reference data obtained from another testing laboratory are valid for the population being tested. Reference values in MSAFP testing consist of a set of median values (pooled from the three testing sites) calculated for each week of gestation using the pooled laboratories MSAFP assay values, measured preferably on the population to be tested with the device. Individual MSAFP test results are then expressed as multiples of the unaffected population median (MOM), which is obtained by dividing each individual MSAFP value by the median value for the relevant gestational week. Strategies are outlined below for establishing median values for both maternal serum and amniotic fluid.

3. Number of investigators:

   Use at least three independent investigators at separate (geographically diverse) sites with at least one in the United States.

4. Sample size:

   Plan a sample size that will be statistically sufficient to determine whether or not the device is safe and effective prior to beginning the clinical trial.

5. Sample type:

   Include data to support use of the test with all claimed specimen matrices (clinical and analytical). Due to interference of anticoagulants with assay results, plasma is not a recommended specimen.

6. Sensitivity and Specificity:

   Regardless of the sample size, present the device's diagnostic sensitivity (true positives) and its specificity [25] (true negatives) and their 95% confidence intervals in the Performance Characteristic section of the product package insert.

7. Sampling method:

   Describe sampling method used in the selection and exclusion of patients. All statistical analysis is based on the "random sample" assumption (e.g., probability sampling).

8. Pooling of investigator's data:

   Present clinical information and data with analyses and conclusions by each investigator. Additionally, present pooled data from each of the investigators, if statistically and clinically justified.

9. Describe statistical methods used and provide confidence intervals.

10. Representative data:

    Present the clinical information and data from the targeted population which must represent the gestational ages for which the device is intended.

11. Establishing reference median values in maternal serum:

    Present validated median values to the population being recommended for testing because of factors such as variability in reliability of gestational dating. Proficiency testing programs find that MSAFP measurements vary by as much as 15% between laboratories, even when results are expressed in IU/mL.[80] The major contributing factor to explain these differences is a bias among manufactured AFP devices. The use of median values obtained from published sources, as reference data is therefore, contraindicated.

12. Establishing reference median values in amniotic fluid:

    Obtaining sufficient numbers of amniotic fluid samples may be difficult for manufacturers. For example, a manufacturer may test 3,000 women per year, identifying only 2 to 4% of these women as candidates for amniocentesis. Amniotic fluid samples sent for AFP

analysis by cytogenetic laboratories may be used to supplement those obtained via the AFP testing program, since nearly all such specimens will be from unaffected pregnancies. FDA recommends that for each gestational week for which the kit is recommended, 50 amniotic fluid samples be used in calculating the median used for interpreting amniotic fluid samples from a particular maternal patient.

Amniotic fluid AFP values are expressed as multiples of the median in the same way as described for maternal serum. The relationship of the median values and gestational age is also log-linear for gestational weeks 15-21 (gestational weeks recommended for testing by FDA), but amniotic fluid AFP median values decline rather than increase with gestational age..

B. Monitor Clinical Trials Testing

1. It is important for manufacturers who sponsor clinical investigations to monitor the clinical performance of their testing programs by tracking the number of fetal malformations detected and missed. Affected pregnancies detected must be followed to the birth of the child or elective termination of the pregnancy. Monitoring the initial percentage of women with elevated (positive test results) AFP levels is a portion of FDA required clinical studies (3 separate testing sites of 1000 maternal patients each at geographically diverse testing sites). FDA's rationale for this epidemiologic surveillance is that the initial percentage of women with positive test results affords the clinical trials investigator an opportunity to properly offer genetic counseling prior to the offering of amniocentesis. FDA recommends that successive diagnostic modalities of ultrasonography dating of the fetus, correction of the AFP MOM interpretation (positive test result or negative), including interpretation of AFP levels from amniotic fluid samples and confirmatory testing for the presence of neural acetylcholinesterase must be accomplished with genetic counseling of the maternal patient. As an example, a clinical investigator's testing site laboratory whose MSAFP testing cut-off is at 2.0 MOM would have an initial positive test result rate of 3 to 5%. If the cut-off is at 2.5 MOM as recommended by FDA, the initial positive test result rate should be 1 to 3%. Manufacturers must followup all positive maternal patients with elevated AFP tests with genetic counseling and other maternal care modalities at each of the three testing sites.

2. The manufacturer should be aware that the initial positive test result is very sensitive to changes in precision and accuracy of the AFP assay, long term assay drift, and inappropriate normative reference data (AFP median values).[76] From AFP Post-Approval Studies, FDA found that epidemiologic

monitoring was a powerful addition to traditional quality control procedures and should be an integral part of the manufacturer's clinical testing program. A preliminary report of these studies was presented by Hybritech, Inc., Division of Eli Lilly Company.[79]

For manufacturers and sponsors of AFP test kits, the testing protocol previously referred to and recommended by FDA was published in the November 7,1980 FEDERAL REGISTER[38]. A clarification of the testing protocol and the importance of adequate followup to positive serum AFP tests was published in Clinical Chemistry News, September 1986[42]. The FDA-recommended protocol seeks two consecutive MSAFP measurements, sampled at least 1 week apart, before further diagnostic modalities are suggested. Ultrasonography is performed as the next step to corroborate gestational date, check for twins, evaluate the viability of the fetus, and possibly detect anencephaly. If the elevated MSAFP value remains unexplained, level 2 ultrasonography or gray-scale sonography is performed to search for other fetal malformations, most prominently open spina bifida and open wall defects. Amniocentesis for the purpose of measuring amniotic fluid AFP (confirmatory testing of amniotic fluid samples determined to have elevated levels of AFP are performed for the presence of neural derived acetyl-cholinesterase) is also offered, following genetic counseling of the maternal patient with the attending physician.[36] Generally speaking, the rate of amniocentesis should not exceed 3 percent of the tested population.[62 63]

3. The reason for the determination of the performance characteristics discussed below (for <u>in-vitro</u> devices) are two-fold; to assess the influence of disease prevalence upon the clinical laboratory test kit's assessment of a patient's condition and to assess the clinical laboratory procedures by which the poor detecting power of an <u>in-vitro</u> device for relatively low prevalence disease may be improved. These assessments include clinical sensitivity (CSE) and clinical specificity (CSP). Four parameters help FDA assess the probability of a correct <u>in-vitro</u> device result: sensitivity, specificity, prevalence of disease condition and efficiency of the test results in detecting diseased individuals. Predictive value of a positive test and predictive value of a negative test are secondary performance characteristics which are functions of CSE, CSP. Disease prevalence can and should be calculated for the range of expected disease prevalence. Interpretations of clinical in-vitro devices, aside from clinical considerations, are based on the probability that the test result will be within a given normal range of analyte values; in this instance, the range of elevated values for AFP in maternal specimens derived from pregnancies with neural tube defective fetuses.

Definitions:

Additional definitions are needed to allow interpretive reporting[21 22 23 24 25] of clinical laboratory AFP device results.

False-positive Rate: FDA accepts two definitions of false-positive rate (FPR). In evaluating the overall performance of clinical investigators, a manufacturer's clinical studies manager is interested in the testing laboratories' proportion of disease-free persons tested who test positive (FP) compared to that portion of disease-free persons tested who test negative (TN); for the testing laboratory, the false-positive (FP) rate is

$$FP/(FP + TN)$$

On the other hand, a clinical investigator, and particularly the physician's patient who has already tested positive, is much more concerned with the ratio between false-positive test results and all positive test results, because this ratio relates to the pregnancy outcome of the particular case in question. In this approach, the false-positive (FP) rate is:

$$FP/(FP + TP)$$

This is equivalent to 1-Predictive Value of Positive Test (1-PVPT). In practical situations employing tests for low prevalence diseases or conditions, this latter (detection) definition of false positive rate gives rates that are much higher. It is this definition of the false positive rate which most manufacturer's Managers of Clinical Testing (MSAFP testing programs and Clinical Investigators) apply when communicating with the clinician and with FDA.

4. Comparison Studies:

   a. Compare the device to at least one device for which there is an approved PMA.

   b. Provide data using three different lots tested in one laboratory.

      Compare results obtained using AFP samples free from interfering substances from 40-100 maternal patients covering the whole assay range (from low to high levels of AFP.[7 10] Analyze the data using linear regression methods [60] (the X axis is the independent variable or comparison test; the Y axis is the dependent variable or new test[54 56]). Linear-regression analysis is often most useful for estimating the differences or errors between two analytical methods, because the errors can be calculated at any medically important concentration

within the range studied; furthermore, the slope and intercept may give some indication of the type of systematic error, which may aid in reducing the analytical error, which may then aid in reducing the analytical errors. Because the reliability of the estimates of slope and intercept can be affected by nonlinearity in the data set, outliers, a narrow range, and variability of the comparison method, it is preferable that samples cover the complete range of concentrations that might be encountered[51]

5. Prozone or High-Dose Hook Effect Studies:

Immunoradiometric (IRMA) and similar type assays:

Test a sample with a very high concentration of AFP, diluted and undiluted.[53] If the test result is not erroneously low, state in the Performance Characteristics section of the product package insert the quantitative level below which no high dose hook effect was observed.

6. Stability Studies

a. Stability study requirements are outlined in 21 CFR 809.10 (a) and (b), 21 CFR 211.166. These studies are performed and conducted with the above specifications and performance evaluations. Real time stability studies should be the basis for estimating expiration dating of reagents. Data from three different manufactured lots are required [53]. Data from accelerated stability studies are acceptable only as interim data.

b. Shipping conditions that must be addressed. Include test results that show that the reagents are stable under variable shipping temperatures or state why the device would not be affected by shipping temperatures, e.g., the device is shipped only on dry ice by guaranteed overnight delivery service.

Labeling Considerations:

Provide the slope, intercept, and their estimated standard errors, correlation coefficient, the standard error of the estimate of the working curve; the assay range, and nature and size of samples tested should be reported in the Performance Characteristics section of the package insert. Copies of all proposed labeling for the device, including any physician and patient brochures, literature, or advertising that constitutes labeling under section 201(m) of the act (21 U.S.C. 321 (m)) must

be provided. Patient brochures must be provided to women with clearly written descriptions of the characteristics of the fetal neural tube conditions. The information presented can obviously not be exhaustive, and it should suggest that further questions be discussed with the physician. The patient brochure should also contain information concerning the procedures available to make a definitive diagnosis and should urge the mother to wait for additional test results before taking further action.

If she is to find the explanation of the follow-up procedures meaningful, the mother must clearly understand the need for them. The most important follow-up procedures, notably amniocentesis and the ultrasonic scan, should be explained in terms of how they help provide the physician with a method of interpreting test results. The general labeling requirements for medical devices are contained in 21 CFR Part 809.10. These regulations specify the minimum requirements for all in vitro devices. Additional guidance regarding device labeling can be obtained from FDA's publication "Labeling: Regulatory Requirements for Medical Devices", and from the Office of Device Evaluation's "Device Labeling Guidance"; both documents are available upon request from the Division of Small Manufacturers Assistance (HFZ-220), Center for Devices and Radiological Health, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

The package insert should be evaluated carefully because the information contained in these seven sections is important for correct interpretation of AFP test results. These seven sections are: (1) intended uses, with particular attention paid to the sponsor's attempts to include "inferred intended use" or expanded applicability based upon "literature citations" with little or no clinical data to support this expanded use, (2) conditions for use, (3) principles of procedure or operation, (4) reagents, (5) limitation(s) of the procedure, (6) interpretation of test results and (7) performance characteristics.

Manufacturers who choose to move the production facilities of their AFP device must re-establish AFP median values by testing 100 serum samples for each gestational week (15 through 20) for calculating median values. Three lots of manufactured reagents should be used to verify performance characteristics of the newly calculated median values. Manufacturers should be sensitive to the fact that clinical laboratories may be tempted to use the median values provided in package inserts as a source of reference data. Such median values have been documented to be widely in error for some manufacturer's kits, resulting in large numbers of false-positive or false-negative test results. FDA requires that manufacturers submit PMA supplements of clinical data to corroborate changes in package insert median values, since use of outdated median values when changes in AFP test kit

reagents (e.g., radioactive iodination of the AFP reagent) may result in large numbers of false-positive or false-negative test result interpretations.

Absolute compliance with the in vitro labeling regulations (21 CFR 809.10) would include patient labeling to give prospective patients (or their parents/guardian) realistic expectations of the benefits and risks of AFP testing. Such information should be written and formatted so as to be easily read and understood by most patients and should be provided to patients prior to scheduling AFP measurement so that each patient has sufficient time to review the information and discuss it with her physician(s). Technical terms should be kept to a minimum and should be defined if they must be used. Patient information labeling should be designed to meet the seventh grade reading comprehension level.

Kearby J. Fugate, Ph.D.

V. BIBLIOGRAPHY

1. Brock DJH, Sutcliffe RG: Alpha-fetoprotein in the antenatal diagnosis of anencephaly and spina bifida. Lancet; ii:197-199, 1972.

2. Leek AE, Ruoss CF, Kitau MJ, Chard T: Raised alpha-fetoprotein in maternal serum with anencephalic pregnancy. Lancet; ii:385, 1973.

3. Brock DJH, Bolton AE, Monaghan JM: Prenatal diagnosis of anencephaly through maternal serum alpha-fetoprotein measurements. Lancet; ii:923-924, 1973.

4. Wald NJ, Brock DJH, Bonnar, J: Prenatal diagnosis of spina bifida and anencephaly by maternal serum alpha-fetoprotein measurement. Lancet; i:765-766, 1974.

5. Brock DJH, Scrimgeour JB: Early prenatal diagnosis of anencephaly. Lancet; ii:1252, 1972.

6. Maternal Serum Alpha-fetoprotein Measurement in Antenatal Screening for Anencephaly and Spina Bifida in Early Pregnancy. Report of the U.K. Collaborative Study in AFP in Relation to Neural Tube Defects. Lancet; i: 1323-32, 1977.

7. Amniotic-Fluid Alpha-fetoprotein Measurement in Antenatal Diagnosis of Anencephaly and Open Spina Bifida in Early Pregnancy. Second Report of the U.K. Collaborative Study on AFP in relation to Neural Tube Defects. Lancet; ii:651-662, 1979.

8. Haddow JE, Macri JN (eds). Proceedings of the First Scarborough Conference: Screening for Neural Tube Defects in the United States, Foundation for Blood Research, Scarborough, Maine, 1977.

9. Haddow JE, Macri JN (eds). Proceedings of the Second Scarborough Conference: Alpha-fetoprotein Serum Screening in Pregnancy, Foundation for Blood Research, Scarborough, Maine, 1978.

10. Haddow JE, Macri JN, Wald NJ (eds). Proceedings of the Third Scarborough Conference: The Regional Application of Alpha-fetoprotein Serum Screening and Ultrasonography in Mid-Pregnancy. Foundation for Blood Research, Scarborough, Maine, 1980.

11. Haddow JE, Macri JN: Prenatal screening for neural tube defects. J Am Med Assoc; 242:515-516, 1979.

12. Wald NJ, Cuckle H: Alpha-fetoprotein in the antenatal diagnosis of open neural tube defects. Br J Hosp Med; 23:473-489, 1980.

13. Kolata GB: Prenatal diagnosis of neural tube defects. Science; 209:1216-1218, 1980.

14. Proceeding, FDA/NICHHD National Conference on Maternal Serum AFP: Issues in the prenatal screening and diagnosis of neural tube defects. July 1980, Washington, D.C., B Gastell,JE Haddow, JC Fletcher, A Neale (eds), U.S. Government Printing Office, 1980.

15. Brock DJH, Barron, L, Jelen P, Watt M, Scrimgeour JB: Maternal serum alpha-fetoprotein measurements as an early indicator of low birth weight. Lancet; ii:267. 1978.

16. Wald N, Cuckle H, Stirrat GM, Bennett MJ, Turnbull AC: Maternal serum alpha-fetoprotein measurements as an early indicator of low birth weight. Lancet; i:268-270, 1977.

17. Macri JN, Weiss RR, Libster B, Cagen MA: Maternal serum alpha-fetoprotein and low birth weight. Lancet; i:600, 1978.

18. Wald NJ, Cuckle H, Stirrat GM, Turnbull AC: Maternal serum alpha-fetoprotein and birth weight in twin pregnancies.Br J Obstet Gynecol; 85:582-584, 1978.

19. Wald NJ, Cuckle H, Stirrat GM: Maternal serum alpha-fetoprotein levels in triplet and quadruple pregnancy. Br J Obstet Gynecol; 85:124-126, 1978.

20. National Committee for Clinical Laboratory Standards. Assessing the quality of radioimmunoassay systems; Revised Guideline. NCCLS document LA1-A. NCCLS, 771 East Lancaster Avenue, Villanova, Pennsylvania, 19085, 1992.

21. Speicher CE, Smith JW:Interpretive reporting in clinical pathology. J Am Med Assoc; 243:1556-1560, 1980.

22. Lundberg, GD: The reporting of laboratory data interpretations: To omit or commit? J Am Med Assoc; 243:1554-1555, 1980.

23. Sohn D: The clinician-laboratory connection, the vital link: Comments regarding the NCCLS proposed standard for clinical laboratory requisition forms. Ther Drug Monit; 1: 1979.

24. Pippenger CE: Editorial. Ther Drug Monit; 1:451-452, 1979.

25. Galen RS, Gambino SR: Beyond normality: The predictive value and efficiency of medical diagnosis. John Wiley and Sons, New York, 1975.

26. Sizaret P, Breslow N, Anderson, SG, and twelve other participants: Collaborative study of a preparation of human cord serum for use as a reference in the assay of alpha-fetoprotein. J Biol Stand; 3:201-223, 1975.

27. Reimer CB, Smith J, Wells TW: The U.S. National Reference Preparation for Alpha-fetoprotein in Mid-Pregnancy Serum. Clin Chem; 28:709-716, 1982.

28. Sizaret P, Anderson SG: The international reference preparation for alpha-fetoprotein. J Biol Stand; 4:149, 1979.

29. Muenz LR, Sizaret P, Bernard C, et al: Results of the second international study on the WHO alpha-fetoprotein standard. J Biol Stand; 6:187-199, 1978.

30. NCCLS Approved Standard: C2-A. Calibration, reference materials and control materials in clinical chemistry. National Committee for Clinical Laboratory Standards, Villanova, Penn. 19085, 1974.

31. Wald NJ, Cuckle H, Boreham J, Stirrat G: Small biparietal diameter of fetuses with spina bifida: Implications for antenatal screening. Br J Obstet Gynecol; 87:20-21, 1980.

32. Weiss RR, Macri JN, Elligers KW: Origin of amniotic fluid alpha-fetoprotein in normal and defective pregnancies. Obstet Gynecol; 47:69 1976.

33. Hook EB: Down syndrome frequency in human populations and factors pertinent to variations in rates. Trisomy 21 (Down Syndrome); Research Perspectives of the National Institute of Child Health and Human Development. DeLaCruz FF, Gerald PS (eds.). Baltimore, University Park Press, 1980.

34. Kjessler B, Johansson SG, Lidbjork G, Sherman MS: Alpha-fetoprotein (AFP) in early pregnancy. Acta Obstet Gynecol Scand Suppl; 69:1-94, 1977.

35. Wald NJ, Cuckle HS, Haddow JE: Should ultrasound be used to estimate gestational age in the screening and antenatal diagnosis of open tube defects in early pregnancy? Lancet; ii:690, 1980.

36. Smith AD, Wald NJ, Cuckle HS, Stirrat GM, Bobrow M, Lagercrantz H: Amniotic fluid acetylcholinesterase as a possible diagnostic test for neural tube defects in early pregnancy. Lancet; i:684-688, 1979.

37. Amniotic Fluid Acetylcholinesterase Measurement in the Prenatal Diagnosis of ONTD. Second Report of the Collaborative Acetylcholinesterase Study. Prenat Diagn; 9:813-829, 1989.

38. 21 CFR Parts 16,20,899 [Docket No. 80N-0002]. FEDERAL REGISTER Vol. 45, No.218, Friday, November 7, 1980, Proposed Rules, AFP Test Kits; Proposed Restrictions and Proposed Additional Quality Control and Testing Requirements. Testing Protocol and Genetic Counseling Flow Chart, page 74167.

39. 21 CFR Parts 16,20,899 [Docket No. 80N-0002]. FEDERAL REGISTER Vol. 48, No.118, Friday, June 17, 1983, Alpha-Fetoprotein Test Kits; Withdrawal of Proposed Rule. Quality Control, page 277781.

40. Haddow, JE and Milunsky, A: Deregulation of Screening For Alpha-fetoprotein In Pregnancy. N Eng J Med; 310:1669, 1984.

41. Burton, BK, Dillard, RG, Clark, EN: The psychological impact of false positive elevations of maternal serum alpha-fetoprotein. Am J Obstet Gynecol; 151:77-82, 1985.

42. Vadlamudi, SK, Fugate, KJ, Appell, RN, and Dierksheide, WD: AFP Testing Protocol Questioned. Letter to Editor: Clinical Chemistry News; 12:no. 9, September 1986.

43. The Quality Control of AFP Reagent and Assay for the Antenatal Screening and Diagnosis of Open Neural-Tube Defects: Report of a (1978) Workshop Sponsored by the U.S. National Institute of Child Health and Human Development, Bethesda, MD. Clin Chim Acta; 105:9-24, 1980.

44. Kallner A, Magid E, and Albert A., eds. Improvement of comparability and compatibility of laboratory assay results in life sciences. Vadlamudi, et al., Third Bergmeyer Conference on Immunoassay Standardization; Lenggries, Germany, Scan J Clin Lab Invest; 51:(Suppl 205):1-143, 1990.

45. Noorgaard-Pedersen B, Toftager-Larsen JP, Hindersson P: Concanavalin-A reactivity pattern of human amniotic fluid AFP examined by crossed affino-immunoelectrophoresis: A definite test for neural tube defect? Clin Genet; 17:1-8, 1980.

46. Ganrot PO: Crossed immunoelectrophoresis. Scand J Clin Lab Invest; 29:39-47, 1972.

47. Dyer SN, Burton BK, Nelson LH: Elevated maternal serum AFP levels and oligohydramnios: poor prognosis for pregnancy outcome. Am J Obstet Gynecol; 31(2):336-369, 1987.

48. Haddow JE, Knight GJ, Kloza EM, Palomaki G: AFP, vaginal bleeding, and pregnancy risk. Br J Obstet Gynoecol; 93:589-593, 1986.

49. Burton BK: Elevated maternal serum AFP (MSAFP)interpretation and follow-up. Clin Obstet Gynecol; 31 (2): 293-305; 1988.

50. Cowan LS, Phelps-Sandall B, Hanson FW, Peterson AG, Tennant L: Prenatal diagnostic center's first year experience with the California AFP screening program. Am J Obstet Gynecol; 160:1496-1504, 1989.

51. Palomaki GE, Hill LE, Knight GJ, Haddow JE, Carpenter M: Second trimester maternal serum AFP levels in pregnancies associated with gastroschisis and omphalocele. Obstet Gynecol; 71:906-909, 1988.

52. Bock JL: Current issues in maternal serum AFP screening. Am J Clin Pathol; 97:541-554,1992.

53. Vadlamudi SK, Stewart WD, Fugate KJ, Tsakeris TM: Performance characteristics for an immunoassay. Scand J Clin Lab Invest; 51:134-138, 1991

54. Peters T, Westgard JO: Evaluation of methods, chapter 7 in: Tietz NW, editor. Fundamentals of clinical chemistry, Third Edition, Philadelphia: Sanders. 225-37, 1987.

55. National Committee for Clinical Laboratory Standards. Evaluation of the linearity of quantitative analytical methods; proposed guideline. Order code EP6-P. 1986.

56. Westgard JO, de Vos DJ, Hunt MR, Quam EF, Carey RN, Garber CC: Method evaluation. American Society of Medical Technology, @@@ Washington. 1978.

57. Information for authors: Clin Chem; 37:1-3. 1991

58. National Committee for Clinical Laboratory Standards. Interference Testing in clinical chemistry; proposed guideline. Order code EP7-p, 1986.

59. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices - second edition; tentative guidelines. 1-56. Order code EP9-p, 1991.

60. National Committee for Clinical Laboratory standards. User Comparison of quantitative clinical laboratory methods using patient samples, proposed guideline, 6 (1). Order code EP9-p, 1985.

61. Bayes Reverend Thomas: An essay toward solving a problem in the doctrine of chance. Philo Trans Roy Soc; 53:370-418. 1763.

62. Adams MJ, Windham GC, James LM, Greenberg F, Clayton-Hopkins JA, Reimer CB, Oakley GP: Clinical interpretation of maternal serum AFP concentrations. Am J Obstet Gynecol; 148:241-254, 1984.

63. Crandall BF, Lebherz TB, Schroth PC, Matsumoto M: Alpha - fetoprotein concentrates in maternal serum: Relation to race and body weight. Clin Chem; 29:531, 1983.

64. Adams MJ Jr, Windham GC, James LM, Greenberg F, Clayton-Hopkins JA, Reimer CB, Oakley GP: Risk reporting of maternal serum AFP (AFP) concentrations. Atlanta: U.S. Department of Health and Human Services, 1985.

65. Wald NJ, Cuckle H: Recent Advances in Screening for Neural Tube Defects and Down's Syndrome, Bailliere's Clinical Obstetrics and Gynaecology, Rodeck C (ed), Vol. I; 3:649-676, 1987.

66. Crandall BF, Robertson RD, Lebherz TB, King W, Schroth PC: Maternal serum alpha-fetoprotein screening for the detection of neural tube defects. Western J Med; 138:524-530, 1983.

67. Macri JN, Kasturi RV, Krantz DA, Koch KE: Maternal Serum AFP Screening, Maternal Weight, and Detection Efficiency, 'Am J Obstet Gynec; 155:758-760, 1986.

68. Soloway HB, Sohn AP, Morris C, Slaughter RJ: Operational Considerations in Maternal Serum AFP Screening, MLO; December 37-40, 1988.

69. Milunsky A, Alpert E, Kitzmiller JL, Younger MD, Neff RK: The Importance of Serum AFP Screening in Diabetic Pregnant Women, Am J Obstet Gynec; 142;1030-1032, 1982.

70. Wald N, Cuckle H, Boreham J, Terzion E, Redman C: The Effect of Maternal Weight on Maternal Serum AFP Levels, Br J Obstet Gynec; 88:1094, 1981.

71. Cowchock FS, Jackson LG: An analysis of pregnancies with elevated alpha-fetoprotein levels in maternal serum and/or amniotic fluid samples, Birth Defects; XV:75-85, 1979.

72. Smithells RW, Seller MJ, Harris R, Fielding DW, Schorah CJ, Nevin NC, Sheppard S, Read AP, Walker S, Wild J: Further experience of vitamin supplementation for prevention of neural tube defect recurrences. Lancet; ii:1027-1031, 1983.

73. Hibbard ED, Smithells RW: Folic acid metabolism and human embryopathy. Lancet; i: 1254, 1965.

74. Laurence, KM, James N, Miller MH, Tennant GB, Campbell H: Double blind randomized controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. B M J; 282:1509-11, 1981.

75. Czeizel AE, Dudas I: Prevention of the first occurence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med; 327:1832-5, 1992.

76. Bishop JC, Dunstan FDJ, Nix BJ, Reynolds TM, Swift A: All MoMs are not equal: some statistical properties associated with reporting results in the form of multiples of the median. Am J Hum Genet; 52:425-430, 1993.

77. Fugate KJ: Post-Approval studies for monitoring the performance of alpha-fetoprotein test kits as an aid in the detection of ONTD. Immunology Device Panel Meeting. Hubert H. Humphrey Building, Washington, D.C. June 22, 1988.

78. 21 CFR Parts 16,20,899 [Docket No. 80N-0002]. FEDERAL REGISTER Vol. 48, No.118, Friday, June 17, 1983, Alpha-Fetoprotein Test Kits: Withdrawal of Proposed Rule. Pages 277780-277782.

79. Felder RA, Butts W, Bradley L, King P, MacMahon W, Wians F,Jr., Dev J: A multi-center study of serum AFP as an aid in the detection of fetal open neural tube defects (NTD). Clin Chem; 36: Abstract; Annual Meeting, July 25, 1990.

80. Knight GJ, Palomaki GE, Haddow JE: Assessing reliability of AFP test kits. Contem Ob-Gyn Tech; October 1987.